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PulseNet News

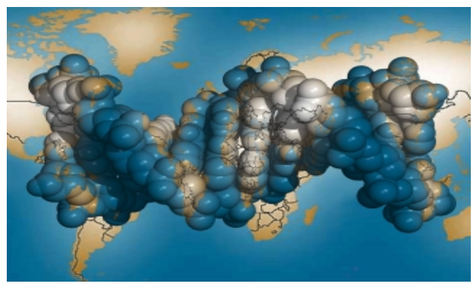
The National Molecular Subtyping Network for Foodborne Disease Surveillance

State & Local Public Health Laboratories in the United States
PulseNet North - Canada

PulseNet, the next generation: DNA sequencing-based subtyping meeting a success.

Article by Andy Sails, Patti Fields, FDDB, CDC, and Shari Rolando, APHL PulseNet Program Manager.

In mid-January, the Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) sponsored a meeting to discuss the development of the next generation subtyping method for deployment in PulseNet laboratories around the country and world. The meeting, held in Decatur, GA, CDC offices, was entitled "PulseNet planning meeting for DNA sequencing-based subtyping." The meeting was designed to guide the research to be performed under the PulseNet contracts that were awarded to three of our participating public health laboratories last year. Our research goal is to ensure that the PulseNet Program is prepared to use future technologies as they become available for enhanced subtyping and tracking of foodborne illnesses. Experts from both public health and academia came together at the meeting to discuss DNA sequencing-based strategies for the epidemiological characterization of foodborne pathogens. Attendees included William "Chip" Beckwith from Connecticut, Sandi Smole from Massachusetts, and Kristen Pederson from Minnesota Public Health Laboratories, the three laboratories that have been funded through APHL to investigate DNA sequencing-based subtyping methods for *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella*. These researchers gained knowledge from, and formed partnerships with several experts in the field of comparative DNA sequencing and related technologies, including: Martin Maiden (Oxford University), Paul Keim (Northern Arizona University), Indira Kudva (Massachusetts General Hospital), Martin Weidmann (Cornell University), Sophia Kathariou (North Carolina State University), Stanley Maloy (University of Illinois), and Michael McClelland (Sidney Kimmel Cancer Center), as well as several researchers from CDC's Foodborne and Diarrheal Diseases Branch (FDDB).



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PUBLICATIONS AND ABSTRACTS

PFGE Diversity among *Salmonella Heidelberg* in the United States. Virgin FW, Kubota K, Ribot EM, Hunter SB, International Conference on Emerging Infectious Diseases, Atlanta, Georgia 24-27 March 2002.

PulseNet Experience: Software Changes and Improvements to Online *E. coli* National Database. Hunter SB, Kubota K, Ribot EM, Swaminathan B. Presented at International Conference on Emerging Infectious Diseases, Atlanta, Georgia 24-27 March 2002.

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Call For Contributions.

The editor welcomes any contribution for the "PulseNet News" Newsletter in the form of short articles, news of recent publications, conference abstracts, news, questions / answers and troubleshooting, new startes and anything else related to PulseNet. Please direct all submissions to the editor (ztu1@cdc.gov).

Question, Answers and Troubleshooting

Experiencing incomplete restriction with enzymes?

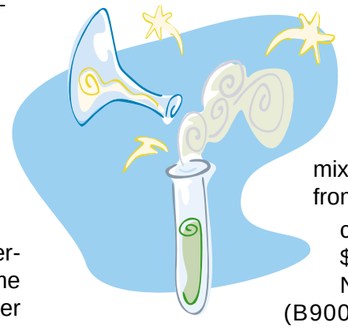
Article by Mary Ann (Lambert) Fair, PulseNet Methods Development and Validation Laboratory, Foodborne and Diarrheal Diseases Branch, Centers for Disease Control and Prevention

What can be done to solve this problem?

A number of PulseNet participants, including CDC personnel, have observed incomplete restriction with some lots of *Xba*I from Roche Diagnostics Corporation. Other participants including Wayne Chmielecki from the Pennsylvania Department of Health, have observed a "washed out" appearance in lanes of some strains of *Salmonella* ser. Newport, which he remedied by adding twice the amount of *Xba*I (100 units per plug slice instead of 50 units). Last year, we noticed incomplete restriction when some strains of *Salmonella* and *Shigella* PFGE plugs were restricted with *Spe*I and *Not*I from Roche, even though we knew there was nothing wrong with the plug preparation because restriction with *Xba*I had given good results. We added 1X Bovine Serum Albumin (BSA) to see if it would improve the restriction. When duplicate PFGE plugs were restricted with and without BSA and run on the same gel, those with BSA had complete restriction, while those without BSA still had incomplete restriction. We are currently conducting experiments comparing the activity of *Xba*I from different suppliers and will report our results at a future date.

Steve Dietrich (Michigan Public Health Lab) recently had difficulties achieving complete digestion with *Xba*I from a new tube of enzyme that was from the same lot (Roche, #92260220) as one that had worked well previously. He talked with Roche technical support when this occurred, but they were not aware of any problems with that lot. Although he resolved the incomplete restriction by adding BSA to the restriction digestion mixture of *Salmonella* and *Shigella* PFGE plugs, the Roche representative said that BSA was not necessary because of the high purity of their enzymes. However, the addition of BSA to the Roche *Xba*I did resolve incomplete restriction that CDC and Michigan had observed with some lots of enzymes from this supplier.

BSA is used to maintain enzyme stability against denaturation and to inactivate components in the enzyme preparation that may inhibit restriction. Some manufacturers such as New England BioLabs supply and recommend using BSA with their restriction enzymes, but Roche does not. It is inexpensive and comes as a 100 X stock solution, which is diluted to 1X in the restriction digestion mixture. BSA can be purchased from a number of companies including Promega (R3961, $16-$20 for 10 mg), NewEngland BioLabs (B9001S, $10 for 25 mg), and Roche (711 454, $57 for 20 mg). These prices are subject to change.



More information concerning this will be posted to the PulseNet Listserv, and there will be at least one presentation about the use of BSA in restriction digestion mixtures at the April 2002 PulseNet update meeting in Ann Arbor. Although all of the problems some people have experienced are not easily explained, they could include:

1) Activity of the enzyme is not optimal due to poor quality control and/or mishandling of the enzyme during shipping or after arrival. Enzymes should not be left out at room temperature, but kept on ice or in a -20^{O}C insulated storage container at all times. If several people in one laboratory are using the same vial of enzyme, the activity of that specific vial could be compromised without others knowing it.

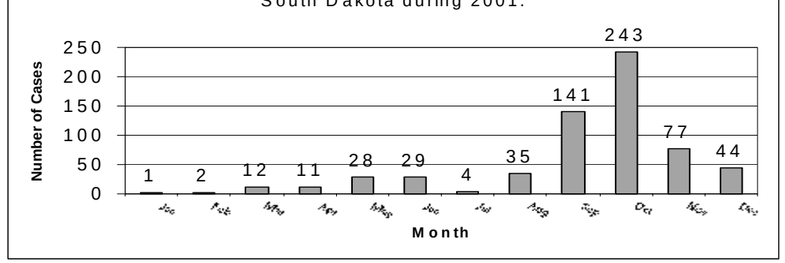
2) Incomplete lysis and/or washing of the PFGE plugs. We have observed incomplete restriction on part, but not all, of the plugs that were made at the same time. When the "problem" plugs were washed 2 more times with TE buffer, the incomplete restriction was eliminated (same vial of enzyme and operator), indicating that not all of the lysis buffer was initially removed from some of the plugs during the washing steps.

Contiune Page 5

Use of PFGE in a *Shigella sonnei* epidemic in South Dakota.

A major outbreak of *Shigella sonnei* infection occurred during 2001 and was the first to occur since the South Dakota State Public Health Laboratory introduced PFGE testing. This was the largest outbreak this state has seen in the past 40 years with the majority of cases occurring in the southwestern part of the state. The first case was reported in January of 2001 with new cases being reported each week thereafter. Figure 1 illustrates the monthly progression of the 627 cases reported up until December 17, 2001. The majority of cases were associated with daycare facilities or with secondary transmission within households and extended families, with little evidence of foodborne transmission. Overall South Dakota had the highest incidence of shigellosis in the U.S. in 2001, at 83 cases per 100,000 population.

Figure 1. Monthly incidence of *Shigella sonnei* cases in South Dakota during 2001.



With no experience working with *Shigella*, I posted a message to the listserv in June to ask for suggestions about how to approach a large outbreak of this nature. The consensus, from more experienced laboratories including the CDC, was that it is not necessary to type all isolates in large outbreaks. The aim of this article is to provide a summary of the results of the first 12 months of performing PFGE on *Shigella* in South Dakota.

After discussing the information obtained from my listserv query with epidemiology staff, we established the following criteria for testing isolates. In cases where the epidemiology was questionable, we typed all of the isolates. In communities where the outbreak was known to be occurring, we selected isolates for typing based on their collection date and their submitting facility so we could monitor potential changes in the outbreak pattern. These criteria required us to test one isolate from each facility approximately every two weeks. We also typed all isolates from communities not previously associated with the outbreak.

Based on our criteria, we typed a total of 181 isolates of *S. sonnei* during the outbreak and recognized 18 different patterns. We identified two major patterns that accounted for 78% of the total cases. There was a single band difference between these two major patterns and sixteen other minor patterns which accounted for the other 22% of the isolates. Minor patterns that were epidemiologically linked to the outbreak often differed from the major patterns by only one or two bands in various positions. In some cases slightly different patterns occurred within cases in the same household.

Monitoring the chain of transmission of *Shigella* can be difficult due to the organism's low infectious dose, the short disease incubation period, and the activities or mobility of the host population. We found PFGE to be very useful in monitoring the progression of this outbreak, with similar PFGE patterns often supporting suspected epidemiological links between cases. Clusters of the same major patterns appeared in new geographic areas, strongly suggesting that cases occurred through transmission of the epidemic strain through some unknown route, rather than emergence of a new outbreak strain. Some cases were excluded from the outbreak partly based on differing patterns, although the cases did occur within outbreak communities. During the course of the outbreak, the antibiotic sensitivity pattern changed with isolates demonstrating increased resistance to trimethoprim-sulfamethoxazole. Interestingly, this change was not associated with changes in PFGE patterns. As this outbreak appears to come to a close it will be interesting to see if a new outbreak pattern emerges in 2002. For this, we will have to wait and see.

Article by Christopher Carlson, Epidemiology and Laboratory Capacity Project Coordinator, South Dakota Public Health Laboratory

Contiune from Page 2, What can be done?

3) Strain-specific reasons:- a number of laboratories (including CDC) that test *S.* ser. Newport have observed that some strains have lighter or "washed out" bands when they are restricted with *Xba*I. In our hands, increasing the cell concentration and/or the lysis time to 4 hours did not improve the results. Using 100 units of *Xba*I improved the appearance of these strains on the gel and eliminated the "washed out" appearance; however, this solution is expensive because twice the recommended amount of enzyme is actually needed. When these "different" *S.* ser. Newport strains were restricted at CDC with 50 units of *Xba*I and 1X BSA, the restriction was satisfactory.

Please let us know at mal3@cdc.gov or PFGE@cdc.gov if your laboratory has observed similar examples with *Xba*I or other enzymes, especially from those companies who supply and recommend the addition of BSA to restriction digestion mixtures. Also, let us know if this recommended solution (or any others) helps your laboratory eliminate incomplete restriction or the "washed out" appearance of bands.

Use of trade names and commercial sources is for identification purposes only and does not imply endorsement by CDC or the U. S. Department of Health and Human Services.

PulseNet Listserv soon to be replaced by PulseNet WebBoard.

Article By Susan Hunter, Microbiologist / Chief, PulseNet National Database Administration Team, CDC

The PulseNet Listserv has been a successful tool to facilitate communication among PulseNet participants and to hasten information to participating laboratories. We now have over 275 people subscribed to the PulseNet listserv, with 205 original postings and 1165 replies during 2001. As the number of participants has increased, so has the volume of messages. PulseNet participants have asked us to decrease the number of email messages that they receive. In order to do this, while still giving participants access to all of the posted information, we will soon be moving to a WebBoard format. The PulseNet WebBoard will be divided into "conferences" with participants being able to post messages to any conference. Participants will also be able to choose to receive individual emails or as daily digest of all the messages posted on each conference. Participants will be able to log on to the WebBoard at any time and review postings in the various conferences. This WebBoard format will considerably reduce the number of emails received by PulseNet participants, yet still provide a forum for the rapid dissemination of information.

ENTER-NET MEETING

Article by Susan Van Duyne, Microbiologist / PulseNet Database Administration Team, CDC

At the end of January, Bala Swaminathan, Susan Van Duyne, together with Chris Braden from the CDC Foodborne and Diarrheal Diseases Branch attended the fourth Enter-net workshop in Athens, Greece. Bala Swaminathan presented "PulseNet Network Successes" and Susan Van Duyne presented "The PulseNet National Database for Salmonella: An Overview." Both presentations were warmly received. Two other presentations related to PulseNet were also given. Helen Tabor from the National Laboratory for Enteric Pathogens (Winnipeg) presented "Relationship between PulseNet North and PulseNet," which explained how the two networks coordinate activities, but remain independent. Geraldine Doran from University College Hospital, (Galway, Ireland) compared the PulseNet standardized protocol for Salmonella with another PFGE protocol previously used in their laboratory. Her TIFF image slides of PFGE patterns obtained with using the two protocols vividly and convincingly demonstrated the superiority of the PulseNet standardized protocol over other PFGE protocol. This glowing endorsement of the PulseNet protocol by a European Union member country was especially important in convincing the attendees to adopt PulseNet protocols instead of developing their own.

At the end of the workshop, the Enter-net project leaders, Enter-net international collaborators, and the members of the European Salm-Gene Project reached an agreement on the establishment of PulseNet Europe. PulseNet Europe will begin operation later this year and will use PulseNet-compatible protocols for DNA "fingerprinting" of Salmonella and Shiga-toxin producing *E. coli* (STEC) to facilitate rapid comparison of the DNA "fingerprint" patterns between North America and Europe. A PulseNet Europe Implementation Committee was formed at the meeting and Peter Gerner-Smidt, Staten Seruminstitut, Copenhagen, Denmark, was elected as the chair of the committee. We invited the Enter-net project leaders and collaborators to participate in the 6th Annual PulseNet update meeting, Ann Arbor, Michigan, April 8-10, 2002.

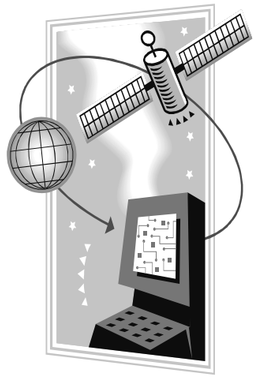
For further information on Enter-Net visit, http://www.phls.org.uk/International/Enter-Net/enter-net.htm

PULSENET STAYS CONNECTED.

Article by Shari Rolando, APHL PulseNet Program Manager

In January 2002, PulseNet area laboratories and representatives from CDC and AHPL participated in a conference call, which heralded a new era in communication within the network. Each area lab reported some of the highlights of their activities during 2001, such as publications, outbreak investigations, staffing changes, multi-state collaborations, and technical advances. Staffing changes figured prominently in the discussions with many of the area labs reporting a significant turnover during the past year, resulting in increased training needs for the new staff. It was emphasized that APHL and CDC need to be informed of staffing changes within the labs, especially if there is a change in the person submitting gel images to the database team at CDC.

In addition, APHL has initiated a calendar of regional bi-monthly conference calls to promote communication between the regional and area labs. The aim of these calls is to promote increased collaboration between the state labs, facilitate regular outbreak investigation discussions, and provide an outlet for the dissemination of technical information. APHL plans to distribute conference call bridge information and agendas prior to each call. In addition, representatives from CDC and APHL will also be invited to participate in the calls.

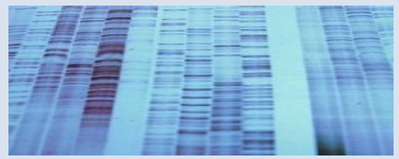


Finally, additional conference calls, which may be regional or national, will address specific technical issues and may include representatives from external companies such as Applied Maths or BioRad. The first of these calls is scheduled for March 2002 and will include Lem Del Rosario of Applied Maths who will answer previously collated questions and participate in online discussions.

Continue from page 1, The Next Generation

Rob Tauxe, Chief of FDDB, CDC and Shari Rolando (PulseNet Program Manager, APHL) welcomed the researchers to the meeting. Bala Swaminathan (Chief, FDDB, Laboratory Section, CDC) opened the first session by describing the current status and possible future of PulseNet. His presentation emphasized the importance of developing the next generation of subtyping methods for PulseNet noting that the predicted useful life of PFGE is 8-10 years. He cautioned that new methods must not only be as good as PFGE, the current 'gold standard', but must provide additional benefits. These new methods must be more sensitive, provide more informative results with less ambiguity; the methods must also be more rapid and amenable to automation and to facilitate easier comparison of results between laboratories. These criteria became the 'mantra' of the meeting with discussions focusing on which method or combination of methods might fulfil these demanding criteria. Finally, John Threlfall of the United Kingdom's Public Health Laboratory Services (PHLS) and Clifford Clark of the National Microbiology Laboratory provided an international perspective for future subtyping needs.

The next session provided an overview of three DNA-based subtyping methodologies. Martin Maiden described multi-locus sequence typing (MLST), Paul Keim described variable



number tandem repeat typing (VNTR), and Indira Kudva introduced the delegates to the polymorphic amplified typing sequences (PATS) method. The morning ended with Andy Sails (FDDB, CDC) describing ongoing CDC research investigating approaches for DNA sequencing-based subtyping of Campylobacter jejuni. The rest of the meeting focused on three pathogens *L. monocytogenes*, *Salmonella*, and *E. coli* O157:H7. Researchers discussed possible approaches to the subtyping of each of these organisms and identified research plans for each organism.

The overall conclusion of the two days of discussions was that a single method might not fulfill the criteria for an improved typing method set at the beginning of the meeting. However, it was agreed that a coordinated combination of the available methods, such as direct sequencing of specific loci and characterization of VNTRs, may provide both short-term (epidemic) and long-term epidemiological information. It is hoped that the information presented at this meeting will help the three state public health laboratory investigators plan their research for the coming year. Chip, Kristin, Sandy, and FDDB laboratory staff plan to have further discussions at the April, 2002 PulseNet update meeting in Ann Arbor, Michigan.